

## SHORT COMMUNICATIONS

# MOVEMENT OF SPERMATOOA IN VISCOUS ENVIRONMENTS

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Observations of the effects of increased viscosity have been important in attempting to understand the mechanical forces and molecular mechanisms involved in cell motility. Berg & Turner (1979) have recently demonstrated that the movement of bacteria at increased viscosities depends on the nature of the macromolecules used to obtain increased viscosities. With methyl cellulose, an unbranched, long-chain polymer, decreases in rotation rates of tethered *E. coli* were much less than with Ficoll, a highly branched polymer, even though the viscosities measured at the macroscopic level were similar. At the microscopic level, methyl cellulose apparently forms a loose, quasi-rigid network in solution, which is easily penetrated by particles on the scale of bacteria and their flagella, but makes a substantial contribution to the macroscopic viscosity of the solution even at concentrations where 'non-Newtonian' behavior is minimal. This observation has led us to re-examine earlier measurements on the effects of viscosity on the movement of sperm flagella, which were made using methyl cellulose to increase the viscosity (Brokaw, 1966; Brokaw & Simonick, 1977).

Measurements were carried out using ATP-reactivated, Triton-demembrated spermatozoa from the sea urchin *Strongylocentrotus purpuratus*, which were beating their flagella while their heads were firmly attached to the microscope slide surface. Conditions were the same as in earlier experiments (Brokaw & Simonick, 1977), except that the temperature was 18 °C, and polyethylene glycol was omitted from the reactivation solutions because turbidity developed in solutions containing both Ficoll and polyethylene glycol. A viscosity range of 1.1 to 8.0 cP (1 cP = 1 mPa.s) was usable; the measurements of Berg & Turner (1979) on the rotation rates of tethered *E. coli* covered the range of 0.8 to 5 cP. Measurements of the beat frequencies of attached sea urchin sperm flagella are shown in Fig. 1 as a function of viscosity. These measurements indicate that, at comparable macroscopic viscosities, the reductions in beat frequency obtained with Ficoll solutions are about 45% greater than those obtained with methyl cellulose solutions. If the measured viscosities of the methyl cellulose solutions are corrected for shear rate differences as in earlier work (Brokaw, 1966), the difference decreases to about 40%. These differences are much less than the factor of three difference in effects on rotation rates of *E. coli* in solutions of Ficoll and methyl cellulose observed by Berg & Turner (1979). Nevertheless, if these results are extrapolated to higher viscosities, they suggest

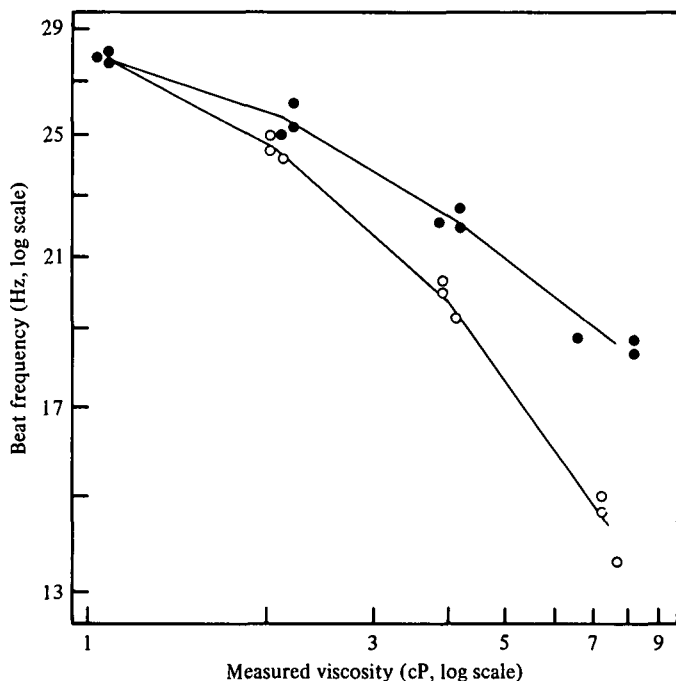


Fig. 1. Beat frequencies of reactivated spermatozoa beating with their heads attached to a microscope slide. Open points are from solutions with Ficoll (Sigma F4375; approx. 400000 m.w.). The solid points are from measurements with methyl cellulose (Fisher M281; 4000 cP) except at the lowest viscosity where neither Ficoll nor methyl cellulose was present. The  $\text{MgATP}^{2-}$  concentration was 0.3 mM and each point represents an average of 40 measurements. Capillary viscometers were used to measure the viscosities of solutions; the highest viscosities correspond to approximately 12.5 % Ficoll or 0.3 % methyl cellulose.

Table 1. *Measurements from photographs of reactivated sperm flagella*

Solution	Sample size	Viscosity (cP)	Frequency* (Hz)	Wavelength* ( $\mu\text{m}$ )
1. 0.3 % methyl cellulose, 0.3 mM $\text{MgATP}^{2-}$	13	6.6	$20.1 \pm 1.4$	$19.6 \pm 1.5$
2. 12.5 % Ficoll, 0.3 mM $\text{MgATP}^{2-}$	27	7.0	$15.3 \pm 1.0$	$18.7 \pm 1.1$
3. 9.2 % Ficoll, 0.3 mM $\text{MgATP}^{2-}$	22	4.7	$20.8 \pm 1.6$	$21.0 \pm 1.3$
4. 0.3 % methyl cellulose, 0.15 mM $\text{MgATP}^{2-}$	35	6.7	$14.9 \pm 1.1$	$21.5 \pm 1.8$
5. No methyl cellulose or Ficoll, 0.3 mM $\text{MgATP}^{2-}$	7	1.1	$31.1 \pm 0.7$	$27.7 \pm 2.0$

\* Mean  $\pm$  S.D.

that previous observations of sperm motility in methyl cellulose solutions having apparent viscosities of up to 80 cP (Brokaw, 1966) may have significantly over-estimated the viscous resistances actually experienced by the sperm flagellum at high viscosities.

During these measurements, we observed that the spermatozoa swimming in Ficoll solutions maintained wave amplitudes better than those swimming in methyl cellulose solutions, and did not appear to show the lower wavelength which might be expected if the lower beat frequency in Ficoll was a result of a higher viscosity (Brokaw, 1966; Brokaw, 1975). We therefore carried out another series of experi-

ments involving photographic measurements of wavelengths (Table 1). Under conditions comparable to the highest viscosities examined in Fig. 1, the solution containing Ficoll (Solution 2, Table 1) gives a wavelength which is only 4.6% less than that obtained with methyl cellulose (Solution 1). If Solution 2 is compared with Solution 3, which has a lower Ficoll concentration such that the beat frequency is about the same as in the highest-viscosity methyl cellulose solution (Solution 1), the wavelength in Solution 2 is about 11% less than in Solution 3. Therefore the wavelength difference between the methyl cellulose and Ficoll solutions at the highest viscosity is significantly less than is to be expected if the beat frequency difference is entirely caused by a difference in viscosity between these two solutions. (The wavelength difference between Solutions 1 and 3 is significant at the 99% level by a standard 't' test.) These measurements suggest that part (perhaps slightly more than half) of the difference between the effects of methyl cellulose and Ficoll on flagellar beat frequency observed in the experiments shown in Fig. 1 may be the result of effects other than a difference in microscopic viscosities. Osmotic modification of the diameter of the axoneme is one possibility (Maughan & Godt, 1979).

Another comparison was made between Solution 2, the high viscosity Ficoll solution, and Solution 4, a high viscosity methyl cellulose solution with ATP concentration lowered to give a beat frequency similar to that obtained with Solution 2. There is a 13% difference between the wavelengths measured in these two solutions (significant well beyond the 99.99% level). Since wavelength is in general insensitive to chemical agents which influence the motility of reactivated flagella (Gibbons, 1974), although it does change indirectly when the beat frequency is changed (Brokaw, 1975), this wavelength difference is probably a true reflexion of a difference in microscopic viscosity between the two solutions with similar macroscopic viscosities.

We conclude that the microscopic peculiarities of methyl cellulose solutions are less important in studies of sea urchin sperm motility than in studies of bacterial motility. There are many possible reasons, which we have not attempted to explore. Methyl cellulose may continue to be a useful agent for obtaining increased viscosities for experiments on motility at the scale of eukaryotic cells, although a need for caution in interpreting the results quantitatively is indicated by our results.

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